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TI ApoE deficiency: Gene replacement using adenovirus vectors.
AU ***Kashyap, V. (1)*** ; Santamarina-Fojo, S.; Brown, D.;
Applebaum-Bowden, D.; Meyn, S.; Parrott, C.; Maeda, N.; Brewer, H. B., Jr.
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0712

ApoE Deficiency: Gene Replacement Using Adenovirus Vectors

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ApoE-deficient (apoE def) mice are a useful model for evaluating the potential for gene therapy. We have generated a recombinant adenoviral vector containing human apoE cDNA (rAdV) for injection of apoE def mice (n=8) with pre-tx lipids: TC = 609 ± 108mg/dl, TG = 101 ± 50mg/dl and chol-rich VLDL/IDL present on FPLC. After IV infusion of either 10^7 or 10^8 pfu, apoE-def mice had peak (day 6) plasma h-apoE levels of 2.3 mg/dl and 648 mg/dl, respectively. Western blot revealed normal sized h-apoE. Expression of these two different levels of apoE in plasma resulted in markedly different lipoprotein changes. Mice achieving physiologic apoE levels (2.3 mg/dl) normalized their lipids (TC = 109 ± 19mg/dl, TG = 56 ± 29mg/dl) at days 4-8; FPLC was normalized with loss of all VLDL/IDL and generation of HDL. Animals with 200X increase in apoE had a biphasic lipid response with initial decrease in TC (230mg/dl) but increase in TG (652 mg/dl); FPLC shifted from chol-rich VLDL/IDL to Tg-rich LDL remnants. By 8-12d apoE (<10mg/dl) and TG (66 ± 40mg/dl) decreased and FPLC revealed a normal HDL profile.In summary:1)We have achieved physiologic replacement (2.3mg/dl) as well as marked overexpression (>500mg/dl) of h-apoE in apoE def mice 2)Physiologic levels of h-apoE normalized plasma lipids 3)ApoE overexpression resulted in transient formation of TG-rich remnants possibly due to high apoE levels blocking receptor mediated remnant clearance 4)Successful replacement of apoE in apoE-def mice demonstrates the feasibility of gene therapy in human apolipoprotein deficiencies.

0713

Lack of Apolipoprotein E does not Affect Bile Acid Metabolism and Bile Secretion of Cholesterol in Chow-Fed Mice.

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Bile secretion of cholesterol (CH), either as such or after its conversion into bile acids, is the major route for removal of CH from the body. Adaptation of bile acid synthesis and of CH secretion represent key metabolic responses in the regulation of plasma CH levels in animals as well as in humans. It has been suggested that apolipoprotein E (apoE) phenotype affects these processes, however, by as yet undefined means. To rigorously evaluate the role of apoE in the regulation of biliary CH removal, we studied bile acid metabolism and CH secretion in homozygous apoE-deficient (apoE -/-) mice kept on normal lab chow. For bile collection, the gallbladder of male mice was cannulated after ligation of the common bile duct. Plasma CH levels were significantly (9-fold) higher in apoE -/- mice than in controls (apoE +/+), whereas plasma triglycerides were similar in both groups. Plasma lathosterol (36-fold) and the ratio lathosterol/CH (4-fold) were higher in apoE -/- mice, indicative for increased CH biosynthesis. However, neither basal biliary bile acid secretion, bile acid pool size and composition nor bile acid synthesis rate differed between apoE -/- and +/+ mice. Likewise, biliary CH secretion was unaffected in the knock-out animals. Our results demonstrate that a lack of apoE by itself does not affect bile acid synthesis and biliary CH secretion, indicating that the uptake of apoE-containing lipoprotein-remnants by the liver does not exert a regulatory role in this respect under the dietary conditions employed.

0714

Apolipoprotein E-Deficient Mice Created by Systemic Antisense Oligonucleotides Administration: A New Model for Lipoprotein Metabolism Studies

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New insights in apolipoprotein metabolism have been gained by the development of transgenic models. As an alternative experimental strategy, we have created apolipoprotein (apo) E-deficient mice created by systemic administration of antisense apo E oligonucleotides (ODN) using HVJ-liposome as a delivery system. ODN contained in a liposome complex containing the HVJ viral envelope was administered into mouse tail vein. Using FITC-labeled ODN, fluorescence was detected in liver, spleen and kidney that was sustained up to 2 weeks after transfection. In contrast, FITC-ODN alone and FITC-ODN in liposome without HVJ showed rapid disappearance of fluorescence (within 1 day). Using the HVJ liposome method, we transfected antisense apolipoprotein (apo) E ODN by intravenous administration which resulted in a marked reduction of apo E mRNA level in liver, but no change in apo B and beta-actin mRNA levels. In mice fed a normal and high cholesterol diet, transient increases in cholesterol and triglyceride levels was observed in antisense apo E treated group, but returned to normal levels. Neither scrambled nor mismatched ODN resulted in an increase in cholesterol and triglyceride. Repeated or cumulative injection of apo E antisense ODN resulted in a sustained increase in cholesterol and triglyceride. Finally, multiple injection of antisense apo E ODN resulted in a chronic hypercholesterolemia and hypertryglyderidemia in mice. The creation of apolipoprotein-deficient mice by intravenous administration of antisense ODN with the HVJ-liposome method is a promising new approach to create animal models to study the role of apolipoprotein metabolism in vivo and for the development of new drug therapy strategies targeting apolipoprotein expression.

0715

Transgenic Mice Expressing Human Apo B and Human Cholesteryl Ester Transfer Protein have a Lipoprotein-Cholesterol Profile Similar to that of Normolipedamic Humans.

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To develop a murine model for studying lipoprotein metabolism and atherogenesis, transgenic mice expressing both human cholesteryl ester transfer protein (hCETP) and human apo B have been developed. Mice expressing hCETP (apo A1 promoter) were bred with human apo B transgenics we previously generated to obtain offspring expressing both transgenes. These chow-fed animals had 3 fold higher CETP activity than humans and expressed human apo B at approximately 50 mg/dl. Compared with non-transgenic (ntg) animals, these animals had increases in total cholesterol (146+/-14 vs. 99+/-11mg/dl) and triglyceride levels (156+/-47 vs. 68+/-25mg/dl) and decreases in HDL (31+/-8 vs. 60+/-11mg/dl) and apo A1 levels (approximately 57 vs. 130mg/dl). The percentage of total cholesterol in HDL, LDL, and VLDL as determined by superose 6 chromatography was 24.1+/-3.7, 65.4+/-5.9, and 10.6+/-3.9, repectively, very similar to that seen in a normal human (28.3, 63.5, 8.2, repectively). Thus, by expressing both hCETP and human apo B, the lipoprotein-cholesterol profile of a chow-fed mouse was transformed into one that resembles the human profile.

0716

Transgenic Mice Expressing High Plasma Levels of Human Apolipoprotein B Develop Severe Atherosclerotic Lesions in Response to a High-fat Diet.

Deborah A. Purcell-Huynh, Robert V. Farese, Jr., Laura M. Flynn, Vincenzo Pierotti, Dale L. Newland, Howard Fein, MacRae F. Linton, David A. Sanan, and Stephen G. Young, Gladstone Institute of Cardiovascular Disease, University of California, San Francisco, CA

We previously generated transgenic mice expressing human apo-B. To assess their susceptibility to atherosclerosis, we analyzed transgenic and nontransgenic mice fed either a chow diet or a diet high in fat (15%) and cholesterol (1.25%). On a chow diet, female transgenics had human apo-B100 levels equivalent to those in humans (~60 mg/dl) and had high levels of LDL cholesterol. When the transgenics were fed a high-fat diet, the human apo-B100 levels in the plasma were unchanged but the *total human apo-B* levels increased by 30%, reflecting increased apo-B48 levels. The transgenics on the high-fat diet had higher total cholesterol levels than the chow fed animals (312 *vs.* 144 mg/dl) and lower HDL cholesterol levels (29 *vs.* 60 mg/dl); nontransgenics on the high-fat diet also had higher cholesterol levels (230 vs. 103 mg/dl) and lower HDL cholesterol levels (37 vs. 67 mg/dl). On the high-fat diet, non-HDL cholesterol levels were significantly higher in the transgenics than in the nontransgenics (283 *vs.* 193 mg/dl), reflecting much higher LDL levels in the transgenics. After 18 weeks of diet, neither the chow fed transgenics nor the chow fed nontransgenics had significant lesions in the aorta. However, transgenics on the high-fat diet had extensive atherosclerotic lesions throughout the ascending thoracic aorta (~100,000 μm^2/section). Nontransgenics on the high-fat diet exhibited ~10-fold smaller lesion areas, with the lesions largely confined to the region near the aortic valve. We conclude that high levels of human apo-B and LDL cholesterol cause extensive murine atherosclerosis, but only in the setting of high fat feeding.

0717

Generation of Mice that Synthesize Apolipoprotein B48, but not Apolipoprotein B100, by Gene Targeting

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In mammals, there are two forms of apolipoprotein (apo) B: apoB100 and apoB48. In humans, apoB100 is made by the liver; apoB48 is made by the intestine and results from editing of the apo-B mRNA, which converts the codon specifying Gln-2153 to a stop codon. To elucidate the physiologic purpose for the two forms of apoB, we used gene targeting in mouse embryonic stem (ES) cells to generate mice that synthesize only apoB48. To accomplish this, we constructed an "in-and-out" sequence insertion vector in which the apoB48 editing codon was mutated to a stop codon. Using the "in and out" selection strategy, we obtained several targeted ES cell clones that contained only the apoB48 point mutation, one of which yielded germline transmitting chimeras upon blastocyst injection. Mice heterozygous for the "B48-only" mutation have plasma lipid levels that are not significantly different from wild-type mice. Unlike the homozygous apoB knockout mice that we generated previously, "B48-only" homozygotes develop normally and are viable, lacking developmental abnormalities of the central nervous system. Sequencing of amplified DNA from homozygotes demonstrated the presence of the apoB48 nonsense mutation, and silver-stained SDS-polyacrylamide gels of the plasma lipoproteins revealed apoB48, but no apoB100. Agarose gel electrophoresis of the plasma of homozygotes demonstrated reduced β-migrating lipoproteins; nevertheless, the lipid levels of homozygotes overlapped with those of wild-type animals. We expect that further metabolic and pathologic studies with these mice will yield new insights into the physiologic purpose for the two forms of apoB in mammalian metabolism and insights regarding the atherogenicity of apoB48-containing lipoproteins.